Contribution from the Chemistry Laboratories of the University of Missouri at Kansas City

Photodynamic Action of Light on Purines (*)

F. Millich and H. Nanaie

Dye sensitized photooxidations are capable of bringing about a more subtle change than ultraviolet radiation or other high energy sources which have been used on nucleic acids and derivatives. Bellin and Oster (1) have been able to deactivate transforming factor DNA by a variety of sensitizing dyes in the presence of light and oxygen. Investigations have shown that the base moieties of DNA are attacked by sensitizer dye. It is our interest to study the chemical consequences of dye sensitized photooxidations, and to characterize the photoproducts.

Irradiation of solutions of purines in phosphate buffer at pH 12 were carred out in the presence of methylene blue and oxygen. White light from a 500 watt tungsten slide projector was used as the energy source, and a cut-off filter was used to exclude radiation of wave lengths below 400 mu. Photooxidation products were separated by thin layer chromatography and were detected by ultraviolet light.

Guanine, xanthine, adenine, and hypoxanthine were irradiated. Guanine and xanthine were photooxidized completely, whereas adenine showed resistance and hypoxanthine remained unaffected. Guanine gave two, xanthine three, and adenine one ultraviolet light absorbing products. One of the photoproducts of guanine was separated in crystalline form when the irradiated solution was acidified. The elemental analysis of a purified sample of this compound gave an empirical formula $C_4N_4O_3H_5$. Though molecular weight has not yet been determined the empirical formula indicates a dimer or higher polymer form. It is slightly soluble in water and has no solubility in common non-polar solvents. It is decomposed at 300°C before melting. It is an acid and forms an insoluble silver salt.

Other sensitizers which were excited with radiation is visible spectral range gave the same two products from guanine. Sussenbach and Berends (2) have reported a similar study using lumichrome as sensitizer, and report finding evidence of four photoproducts, of which they have identified one as guanidine, implying ring cleavage in the purine; we have found but two products, and, further, have found guanidine to be absent. It is not clear that the mercury lamp radiation was properly filtered. The significant difference lies in the fact that sensitization with methylene blue, using red light, limits the energy of activating of this system to a maximum of about 40 kcal/mole, whereas the use of ultraviolet light may involve three times that amount.

The other photoproduct which we isolated from guanine has not yet been purified. It is less thermally stable then the first photoproduct, described above. It may well be a precursor in the procedure to the latter compound, and, in fact, can be converted to the latter compound in a separate experiment. It is our aim to pursue structural identification of these and other photoproducts. We have observed guanosine, for instance, to photooxidize very rapidly under similar conditions.

This work has been supported in part by a National Aeronautics and Space Administration research grant (NsG-651)*

---

(1) J. S. Bellin and G. Oster, Biochem. et Biophys. Acta, 42 553 (1960).

(2) J. S. Sussenbach and W. Berends, Biochem. Biophys. Res. Commun., 16, 263 (1964).